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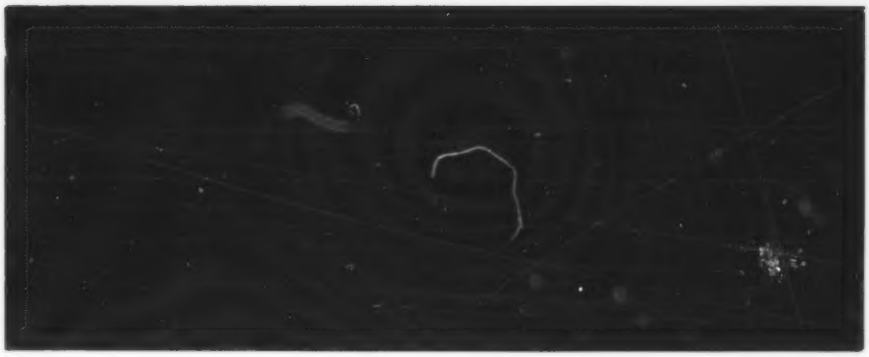

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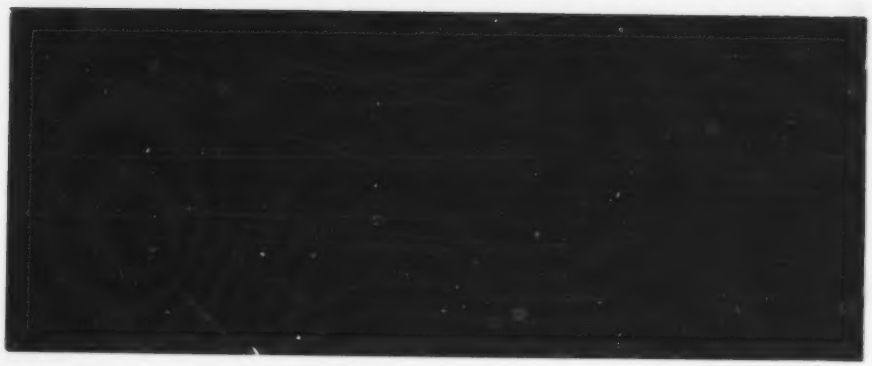

Final Report

2.3-13

Control of Chalkbrood and Other Fungal Problems in Leafcutting Bees

Conducted by
Saskatchewan Alfalfa Seed Producers' Association

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SASKATCHEWAN ALFALFA SEED PRODUCERS' ASSOCIATION

TITLE : Control of Chalkbrood and Other Fungal Problems in Leafcutting Bees

PROJECT NO. : 2.3-13

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Abstract

Surveys of Saskatchewan leafcutting bee populations in 1988 and 1989 have indicated that chalkbrood disease (Ascosphaera aggregata) is present at extremely low levels in the province. Numerous other fungal species have been isolated from leafcutting bee populations at much higher levels. These foliar moulds are potentially harmful not only to leafcutting bees, but also to the health of alfalfa seed producers. Field experiments have demonstrated that foliar moulds contaminating leafcutting bee nest material may be controlled either by dipping in sodium hypochlorite solution or by kiln-heating. Research has also identified the fumigant paraformaldehyde as a highly efficaceous alternative compound for foliar mould control.

SASKATCHEWAN ALFALFA SEED PRODUCERS' ASSOCIATION

CONTROL OF CHALKBROOD AND OTHER FUNGAL PROBLEMS IN LEAFCUTTING BEES

PROJECT 2.3-13

FINAL RESEARCH REPORT

MARCH, 1990

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SASKATCHEWAN AGRICULTURE

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I. ABSTRACT

Surveys of Saskatchewan leafcutting bee populations in 1988 and 1989 have indicated that chalkbrood disease (Ascosphaera aggregata) is present at extremely low levels in the province. Numerous other fungal species have been isolated from leafcutting bee populations at much higher levels. These foliar moulds are potentially harmful not only to leafcutting bees, but also to the health of alfalfa seed producers. Field experiments have demonstrated that foliar moulds contaminating leafcutting bee nest material may be controlled either by dipping in sodium hypochlorite solution or by kiln-heating. Research has also identified the fumigant paraformaldehyde as a highly efficaceous alternative compound for foliar mould control.

II. INTRODUCTION

The alfalfa leafcutting bee, Megachile rotundata, has been used by Saskatchewan alfalfa seed producers as a dependable pollinator of alfalfa for about twenty-five years. At present, over 10,000 ha are devoted to pedigreed alfalfa seed production in Saskatchewan, with another 7,000 ha in common seed production. Sales of leafcutting bee cocoons earn Saskatchewan producers $4 million annually and new grower interest is strong as producers seek to diversify their agricultural operations.

A major problem affecting alfalfa leafcutting bee populations has been chalkbrood disease, caused by the fungus Ascosphaera aggregata. Chalkbrood disease is currently the most serious limiting factor to leafcutting bee populations and alfalfa seed production in the northwestern United States. As a result of this disease, the U.S. has become the major market for Saskatchewan leafcutting bee cocoons. In Canada, chalkbrood disease has become a chronic problem in southern Alberta, with levels as high as 27.8% in some 1988 populations; serious outbreaks of the disease have also been reported in central Manitoba from the 1989 field season. These widespread chalkbrood disease problems increase the probability of accidental introduction of the disease into Saskatchewan leafcutting bee populations. A major outbreak of chalkbrood disease in Saskatchewan would make maintenance of bee populations for alfalfa seed production difficult and would jeopardize the reputation for high quality enjoyed by Saskatchewan bees in the world market.

Also found in association with alfalfa leafcutting bee populations are a number of other fungal species referred to as foliar moulds. These moulds contaminate leafcutting bees, cocoons, and nest material, and have been implicated in human allergic reactions and respiratory diseases. Alfalfa seed producers are exposed to high levels of foliar mould spores and should be made aware of the importance of reducing fungal contamination in leafcutting bee populations in order to minimize contact with potentially harmful spores.

In order to determine the status of chalkbrood disease and other fungal contaminants in Saskatchewan leafcutting bee populations, and in order to develop methods to control these problems, a research project was undertaken with the following objectives:

1. To continue a survey of chalkbrood disease (Ascosphaera aggregata) in Saskatchewan leafcutting bee populations by soliciting bee samples from alfalfa seed producers and by nest-trapping leafcutting bees at sites throughout the province.

2. To monitor levels of other fungal species including foliar moulds and native Ascosphaera species which may adversely effect the viability of leafcutting bee populations.

3. To identify the presence of foliar moulds in leafcutting bee populations which are potentially harmful to the health of alfalfa seed producers.

4. To develop new methods of fungal control for use in decontaminating leafcutting bee nest material and thus enhancing the quality of leafcutting bee populations.

5. To investigate other possible causes of mortality in alfalfa leafcutting bees.

III. RESEARCH METHODS AND RESULTS

A. Saskatchewan Chalkbrood Survey

In order to survey Saskatchewan leafcutting bee populations for presence of chalkbrood disease, samples of bee cocoons were solicited annually from producers throughout the province. Samples were stored at 4°C upon receipt. From each sample, 4 x 15 g subsamples were arranged in polystyrene nest boards and x-rayed with an H.P. Faxatron Unit (14kvp @ 10 sec) using Kodak X-Omat AR film. Exposed x-ray films were then evaluated using a Picker FVS illuminator. Cocoons not containing apparently healthy bee prepupae were noted on the x-ray, dissected, and their contents analysed and tabulated. Following evaluation of samples, data collected were returned to producers. The data summary presented in Table I includes comparative results from the 1985 to 1989 surveys.

The 1988/89 chalkbrood survey (Appendix A) was completed in late April, 1989 with a total of 71 samples received and analysed during the period November, 1988 to April, 1989. A single incidence of classic sporulating larval chalkbrood (Ascosphaera aggregata) was identified in one of the 71 samples submitted to the 1988/1989 survey. This single sporulating chalkbrood cadaver represents 0.002% of all leafcutting bee cocoons evaluated in the survey. Upon detection of this cadaver, work was undertaken immediately to define the extent of the infection in bee populations belonging to the producer in question. A total of 59,479 cocoons were analysed, either in the laboratory or in sessions on site with the producer. No additional sporulating chalkbrood cadavers were identified as a result of this activity. This is the first identification of classic sporulating A.aggregata in Saskatchewan since 1984.

Occurrence of other Ascosphaera species (i.e. those species of this genus which are not classic chalkbrood but which do occur at low levels in some Saskatchewan leafcutting bee populations and which may be pathogenic to leafcutting bee larvae) remained low in the larval category (0.004%) and in the pollen Ascosphaera spp. category (0.5%) for 1988/88. The category of "pollen Ascosphaera spp." refers to native Ascosphaera mould species found growing on pollen balls.

The 1989/90 chalkbrood survey (Appendix B) was completed in late March, 1990 with a total of 76 samples received and analysed during the period November, 1989 to March, 1990. No incidence of sporulating chalkbrood disease was noted in the 1989/90 survey, but a low incidence of apparent nonsporulating chalkbrood was observed.

This form of chalkbrood disease is not well understood; it appears to involve a process of the disease where no spores are formed and thus the disease in this form is not infectious. Levels of non-sporulating chalkbrood have been extremely low over the 1985 to 1989 period of the survey. Populations from which non-sporulating chalkbrood cadavers have been isolated are monitored closely through the chalkbrood survey and also through nest-trapping in the field at selected sites. Occurrence of other Ascosphaera species remained low in the larval category (0.007%) and in the pollen Ascosphaera spp. category (0.6%) for 1989/90.

Table I. 1985 to 1989 Saskatchewan Chalkbrood Survey data (%)

CATEGORY	1985	1986	1987	1988	1989[a]
HEALTHY PREPUPAE	76.1	71.8	76.2	79.6	79.4
DEAD LARVAE/PREPUPAE					
Discoloured	5.0	6.0	4.2	3.9	3.7
Mouldy	0.5	0.6	0.6	0.2	0.3
Ascosphaera aggregata	-	-	-	0.002	-
Non-spor.Chalkbrood	0.002	-	-	0.002	0.009
Other Ascosphaera spp.	0.018	-	0.045	0.002	0.007
MOULDY COCOONS					
Pollen Mould	1.9	5.0	5.4	2.3	2.8
Pollen Ascosphaera spp.	0.5	0.3	1.0	0.5	0.6
PARASITES	0.5	1.5	0.5	2.1	1.8
SECOND GENERATION	0.9	0.5	1.4	0.6	0.9
POLLEN BALLS	12.0	13.1	9.6	9.3	8.9
CRUSHED COCOONS	2.2	1.1	0.7	1.3	1.5
OTHER	0.5	0.2	0.4	0.2	0.3
LIVE COUNT/LB.	3965	3758	3974	4083	4089
LIVE COUNT/KG.	8731	8275	8751	8991	9004
NO. OF SAMPLES	60	42	59	71	76

[a] 1989 data summary based on samples analysed to 31.March.90

A brief overview of other data collected in the chalkbrood survey indicates that the healthy prepupae average, at 79.4%, remained at a high level in 1989/90, while the pollen ball level of 8.9% was at its lowest point during the five year period. Parasite counts were very high at 1.8%, which continues the trend set in the 1988/89 survey.

An examination of 1989/90 statistical data, which are given in Appendix B, indicates that parasite levels ranged as high as 12.7%. In fact, only thirteen of the 76 samples analysed in this survey were found to be free of the chalcid parasite Pteromalus venustus. Larval and pollen moulds, at 0.3% and 2.8% respectively, remained lower than 1986/87 and 1987/88 levels. Other categories, including discoloured larvae, pollen Ascosphaera spp., second generation, and crushed cocoons, remained at levels similar to previous survey results. The increase in percent healthy prepupae in 1988/89 and 1989/90 over previous years appears to be due primarily to a decrease in pollen ball and pollen mould levels. As in 1988/89, pollen ball levels in 1989/90 remained three to four percent below 1985 and 1986 levels, and dead discoloured larvae continued to show a slight downward trend.

In addition to carrying out an extensive annual chalkbrood survey, the monitoring of chalkbrood disease in Saskatchewan leafcutting bee populations was enhanced by placing specially-constructed trapnest boxes at sites in close proximity to alfalfa seed fields throughout the province. Analysis of material collected in these trapnests would help to provide an early warning should an infestation of chalkbrood disease or some other problem occur in a particular area. A series of trapnests was placed in alfalfa leafcutting bee shelters and wild locations at a number of sites in Saskatchewan (Appendix C). Prior to deployment, the wood trapnest boxes were sterilised by heat-treatment and then filled with new polystyrene laminate nest material. The trapnests remained in the field for the entire season and were removed in early September; cocoons were stripped in October and the contents then analysed by x-ray. Information on domestic and wild leafcutting bee species and associated chalkbrood and foliar moulds was thus obtained soon after the end of the field season.

No incidence of chalkbrood disease was found in leafcutting bee cocoon samples collected in trapnests during the 1988 field season. However, in the 1989 field season a single wild leafcutting bee cadaver infected with sporulating chalkbrood disease was identified from a trapnest placed in an alfalfa leafcutting bee shelter. The wild leafcutting bee species involved, Megachile relativa, is a common immigrant into alfalfa leafcutting bee nest material and is the wild species most usually found in conjunction with domestic (M.rotundata) populations. This is the first record of Ascosphaera aggregata infecting Megachile relativa. Subsequent extensive sampling of domestic and wild leafcutting bee cocoons from the location involved indicated no further presence of sporulating A. aggregata in the population.

B. Research on Leafcutting Bee Mycoflora

Data collected in the chalkbrood survey on Ascosphaera species other than A.aggregata (i.e. A.larvis, A.pollenicola, and A.variegata) over the period 1985 to 1989 are presented in Table II.

Table IIa. Occurrence of pollen chalkbrood (1985 - 1989).

Year	No.samples submitted	Samples with chalkbrood no.	%	Average % pollen chalkbrood[a]
1985	60	27	45.0	1.09
1986	42	14	33.3	0.89
1987	59	36	61.0	1.63
1988	71	37	52.1	0.99
1989	76	38	50.0	1.11

Table IIb. Occurrence of larval chalkbrood (1985 - 1989).

Year	No.samples submitted	Samples with chalkbrood no.	%	Average % larval chalkbrood[a]
1985	60	6	10.0	0.23
1986	42	0	0.0	0.0
1987	59	12	20.3	0.22
1988	71	2	2.8	0.15
1989	76	4	1.9	0.14

[a] where (n) is no. samples with pollen or larval chalkbrood.

Data presented in Table II indicate that although pollen chalkbrood levels have remained relatively low over the past five years, a large number of producers do have A.pollenicola and/or A.variegata present on pollen balls in their leafcutting bee populations; as can be observed in the table given in Appendix B,

the level in some populations has been as high as 8.07% in 1989. In sharp contrast, larval chalkbrood data indicate that the level of A.larvis and/or A.variegata on larval cadavers is much less significant than the more widespread occurrence of chalkbrood moulds on pollen and nectar provisions.

Leafcutting bee mycoflora (i.e. foliar moulds, yeasts, and bacteria) other than Ascosphaera species were monitored in bee samples collected in trapnests dployed at various Saskatchewan locations. Mycoflora were sampled using sterile cotton-tipped wood applicators which were wetted with sterile distilled water and mixed about in bags containing cocoons ca 15 sec. The applicators were then placed in sterile screw-top glass culture tubes, reconstituted in 2.0 mL sterile distilled water, vortexed, and plated as 0.1 mL aliquots on potato dextrose agar (PDA). Cultures were incubated at 28°C and assessed at 24 h intervals in order to identify moulds, yeasts, and bacteria present.

In 1988 trapnest samples evaluated, Aspergillus niger and Rhizopus oryzae were the predominant moulds, and Trichosporonoides sp. the most common yeast, identified. Bacterial species cultured included Enterobacter agglomerans, Pseudomonas solanacearum, and Bacillus spp. In 1989 trapnest samples evaluated, predominant mould species identified were Aspergillus niger and the Eurotium/Penicillium spp. complex. Once again, Trichosporonoides sp. was the most common yeast species present, while bacterial species isolated included Enterobacter agglomerans, Bacillus circulans, Flavobacterium breve, and Pseudomonas spp. It appears that dominance among mould, yeast, and bacterial species is variable in any given year and likely is dependent upon factors such as local climate and crop type in the surrounding area.

A complete list of mycoflora isolated from Saskatchewan leafcutting bee populations is given in Appendix D. The presence of these diverse microorganisms in bee populations can often lead to problems with larval development in the bees. Bacteria, in conjunction with yeasts, may ferment the nectar and pollen provisions and thus cause mortality in the leafcutting bee egg or early instar larva. This spoilage process may then lead to overgrowth of the entire cocoon contents by moulds. The concurrent isolation of several mould, yeast, and bacterial species from a single mouldy pollen ball or larval cadaver indicates that all three groups of microorganisms may contribute to leafcutting bee mortality.

C. Health Implications of Foliar Moulds

Many of the moulds found in association with populations of the alfalfa leafcutting bee are potentially harmful not only to the bees, but to alfalfa seed producers as well. An integral part of this research project has been the systematic isolation and identification of moulds associated with leafcutting bee populations. In order to determine the medical status of fungi occurring in the leafcutting bee system, recent medical literature was reviewed for references pertaining to fungal species isolated from Saskatchewan alfalfa leafcutting bees and the nest material in which they work. Table III given below lists a number of foliar mould species which have been associated with human disease processes.

Table III. Some foliar moulds of medical significance which have been isolated from Saskatchewan alfalfa leafcutting bee populations.

Alternaria alternata
Aspergillus glaucus
Aspergillus niger
Cylindrocarpon sp.
Mucor sp.
Penicillium purpurogenum
Penicillium spinulosum
Rhizopus oryzae
Trichoderma sp.
Ulocladium sp.

Aspergillus niger and *Alternaria alternata* have been implicated in allergic reactions and bronchopulmonary disease, and along with *Rhizopus* and *Penicillium* species are important fungal allergens. *Aspergillus* infection is usually acquired via the respiratory tract and may cause problems ranging from pulmonary hypersensitivity disease (e.g.allergy, asthma) to life-threatening infection. *Aspergillus* lung disease may occur regardless of host immune status, although manifestation of the disease is most often seen in the young, the aged, or the immunodeficient individual. Several of the microorganisms listed in the table above are not ordinarily associated with human diseases but under certain conditions may act as opportunistic pathogens.

D. Foliar Mould Control Research

1. Bleach Treatment

Research undertaken previously in this project has indicated that dipping of leafcutting bee nest material in a 3 to 5% sodium hypochlorite bleach solution for 3 to 5 minutes is highly efficaceous for control of foliar moulds. In order to test the efficacy of bleach-spray treatment of polystyrene laminate nest material, an experiment was undertaken at the farm of an alfalfa seed producer in southwest Saskatchewan. Nest boxes were laid flat and sprayed with a 3.0% sodium hypochlorite (NaOCl) bleach solution using a small spray tank connected to a larger, pressure-regulated holding tank (20 -25 psi). The bleach solution was supplemented with Agrol 90, a wetting agent. Nest material was thoroughly wetted, then drained and allowed to dry for seven days prior to field deployment alongside untreated control material. Cocoons were harvested in early September and analysed by x-ray in mid-October (Table IV).

Table IV. Quality of leafcutting bee cocoons from untreated control and bleach-spray treated polystyrene laminate nest material.

Category	Control (%)	Treated (%)
Healthy Prepupae	67.0	77.4
Dead Larvae / Prepupae	4.7	1.3
Mouldy Larvae	0.4	0.1
Pollen Mould	9.6	6.0
Pollen Balls	7.9	8.3
Other	10.4	6.9

The viability of cocoons from bleach-spray treated material was observed to be 10.4% higher than that of cocoons from untreated control nest material. As well, levels of larval and pollen mould were 0.3% and 3.6% lower, respectively, in the cocoons harvested from bleach-spray treated polystyrene laminate nest material. These results indicate that levels of mycoflora in the nest material were substantially reduced by spraying the material with sodium hypochlorite bleach solution prior to field deployment.

2. Heat Treatment

Research conducted previously in this project has shown that use of kiln or macrowave heating is a viable alternative to bleach treatment for the control of foliar moulds, yeasts, and bacteria in leafcutting bee nest material. Following this demonstration of the efficacy of heat treatment at temperatures exceeding 80°C, several alfalfa seed producers in the province have designed and built electric kilns in order to treat wood nest material. At the farm of an alfalfa seed producer in southeast Saskatchewan, wood laminate nest material was treated for a period of ca 7 h at temperatures in the 97°C to 104°C range prior to placement in the field along with untreated control nest material. In the fall of 1988, leafcutting bee cocoon samples from control and treated nest material were analysed for overall quality using x-ray evaluation techniques. Results of this analysis are given in Table V.

Table V. Quality of leafcutting bee cocoons harvested from untreated control and heat-treated wood laminate nest material.

Category	Control (%)	Treated (%)
Healthy Prepupae	81.7	89.3
Dead Larvae / Prepupae	3.2	2.7
Mouldy Larvae	0.4	0.3
Pollen Mould	3.9	0.3
Pollen Balls	8.4	6.6
Other	2.3	0.9

The results of this evaluation show a 7.6% greater level of healthy prepupae in the cocoons harvested from heat-treated wood laminate nest material. Data also show that the pollen mould level is 3.6% lower in the treated nest material cocoon sample. The higher quality of cocoons in the sample harvested from treated material suggests that the viability of leafcutting bee progeny has been significantly enhanced by the use of heat treatment to control mycoflora in wood laminate material.

3. Enilconazole Fumigation

Preliminary research was carried out on the fumigant enilconazole to determine its efficacy as an agent for the control of foliar moulds, yeasts, and bacteria on wood laminate and polystyrene laminate leafcutting bee nest material. Enilconazole is a compound which exhibits antimicrobial activity based on its ability to inhibit synthesis of ergosterol in the cell membrane of a microorganism.

For the application described here, enilconazole was formulated as a fumigant product which is sublimated by burning. Leafcutting bee nest material used in this experiment included wood laminates, milled polystyrene laminates, and moulded polystyrene laminates. Nest material was placed in an isolation room (area 66.9 m^3) at the V.I.D.O. research station in Saskatoon. Tunnels for pre- and post-treatment sampling were selected and marked; pre-treatment sampling was then carried out via standard protocol similar to that reported in Appendix E.

The dose of enilconazole used in this experiment was that recommended in a report from the Veterinary Institute of Croatia (Zagreb), i.e. 5.0 g/35 m^3. The isolation room and its contents were fumigated by lighting the material in two aluminum canisters, each of which contained 5 g enilconazole. Room temperature was ca 24° C and relative humidity ca 40%. After 24 h, the room was entered by an individual wearing a respirator and protective clothing. Post-treatment sampling of tunnels was carried out at this time. Pre- and post-treatment samples were reconstituted in sterile distilled water and aliquots plated on PDA (potato dextrose agar) within several hours of sample collection. Cultures were incubated at 28° C and assessed at 24 h intervals. Data obtained from the analysis of pre- and post-treatment sample cultures are summarized in Table VI.

The level of contamination in the moulded polystyrene nest material was very low and yielded data of limited use which is not included. In wood laminate material, enilconazole fumigation was clearly not efficaceous at the dose tested (ca 0.15g/m^3). Apparent control of a yeast sp. in milled polystyrene material is probably due to inter-tunnel variation in mycoflora.

A number of cocoons containing diapausing leafcutting bee prepupae were also exposed to enilconazole fumigation in this test. It was subsequently determined that mycoflora on the cocoon surfaces were not eliminated. It was also determined that live prepupae were not adversely effected.

Table VI. Efficacy of enilconazole fumigation of nest material.

Type of material	Microorganism	No. cfu pre-treat.	No. cfu post-treat.	% control
Wood Laminate	Moulds	258[a]	388[a]	-
	Yeasts	2645	2521	4.7
	Bacteria	472	528	-
	Total	3375	3437	-
Milled Poly. Laminate	Moulds	161[a]	210[a]	-
	Yeasts	50	0	100.0
	Bacteria	470	1200	-
	Total	681	1410	-

[a] Samples also contained an indeterminate number of Rhizopus cfu.

4) Paraformaldehyde Fumigation

Research on the fumigant paraformaldehyde was undertaken to determine its efficacy for control of foliar moulds, yeasts, and bacteria in leafcutting bee nest material. Paraformaldehyde $(CH_2O)_n$ is a white, crystalline substance available as a powder or prill. It has a melting range of 120-170°C and can therefore be sublimated at relatively low temperatures. A research permit was obtained from the Pesticides Directorate of Agriculture Canada (Research Permit No. 106-RP-88) to allow this research to be carried out under specified experimental and safety guidelines.

Following initial testing which indicated that paraformaldehyde was a promising candidate for use as a foliar mould control compound, samples of used polystyrene laminate (two sources), wood laminate, and polystyrene block leafcutting bee nest material were obtained from four Saskatchewan alfalfa seed producers in spring, 1989. Nest material was placed in an isolation room at the Veterinary Infectious Diseases Organization (V.I.D.O.) research station in Saskatoon and sets of nest tunnels were then randomly selected and marked for sampling.

After a 48 h conditioning period (24.4°C / 78% R.H.), pre-treatment samples were taken and nest material was fumigated with paraformaldehyde at a rate of 20.9 g/m^3. After a 48 h period comprising 24 h passive dissipation and 24 h filtered ventilation, the room was entered and post-treatment sampling undertaken. Pre-treatment and post-treatment sampling of nest tunnels was carried out using sterile cotton-tipped wood applicators wetted with sterile distilled water prior to tunnel sampling and then placed in sterile screw-top glass culture tubes following sampling.

Samples were prepared and cultured in the laboratory within several hours of collection. Applicators used in sampling were reconstituted in 2.0 mL sterile distilled water and vortexed. The samples were then plated as 0.1 mL aliquots on potato dextrose agar (PDA). Cultures were incubated ca 28°C and assessed at 24 h intervals. A complete paraformaldehyde fumigation protocol covering actual treatment and assessment of efficacy is included in this report as Appendix E.

Paraformaldehyde-treated nest material was stored under sterile conditions following treatment and deployed in shelters at four field sites (one type of nest material per location) along with untreated control nest material during June, 1989. Field observations were made during July and August to determine nest material acceptance and rate of tunnel-filling by alfalfa leafcutting bees.

In the fall, when harvesting of leafcutting bee cocoons from nest material was completed, samples of cocoons from treated and control nest material from each site were evaluated by x-ray analysis to determine leafcutting bee sample quality. Following x-ray analysis, cocoons containing healthy leafcutting bee prepupae were selected from each sample and incubated at 28°C in order to assess percent successful emergence and rate of emergence.

An additional efficacy test was undertaken to assess the performance of paraformaldehyde fumigation in controlling foliar moulds under actual field conditions. Nest material was fumigated in a metal granary constructed on a concrete pad and sealed with insulation and caulking at all joints; the roof cap was sealed with plastic sheeting prior to fumigation. Material placed in the granary included field-contaminated polystyrene laminate and polystyrene block nest material and wood leafcutting bee incubation trays. Following a conditioning period of 48 h at 25°C and 70% R.H., pre-treatment sampling and paraformaldehyde fumigation (20.0 g/m^3) were carried out. Post-treatment sampling was done after a seven day period during which paraformaldehyde gas was allowed to passively dissipate. All samples were prepared and cultured according to standard protocol.

Data on control of mycoflora (mould, yeast, and bacterial species) in nest material are given in Appendix F. Detailed information on species of mycoflora identified in pre- and post-treatment sample cultures from various types of nest material is listed in Appendix G (polystyrene laminates) and Appendix H (polystyrene blocks, wood laminates). Evaluation of sample culture data indicates that paraformaldehyde fumigation at a rate of ca 20 g/m^3 is highly efficaceous for control of mycoflora in all nest materials tested, with overall control levels ranging from 86.5% to 99.1%.

Common foliar mould species, including Alternaria, Aspergillus, Eurotium, Penicillium, and Rhizopus spp., were virtually eliminated from polystyrene laminate nest material by paraformaldehyde fumigation. In polystyrene block and wood laminate nest material, overall mould control was very good except in the case of the Eurotium/Penicillium complex. A result of particular significance is that in all nest material with the exception of wood laminates, the mould Rhizopus oryzae, an extremely persistent contaminant, was totally controlled by paraformaldehyde fumigation.

The most common yeast species associated with leafcutting bee populations, Trichosporonoides sp., was very well controlled in all types of nest material (range 93.3% to 99.9%). Control of bacterial contaminants (predominantly Bacillus and Enterobacter spp.) was most efficient in polystyrene nest material, ranging from 90.7% to 99.0%. In wood laminate nest material, however, rate of bacterial control was 67.3%. This lower level of control could be due to the organic nature of wood, which may allow some bacterial species to partially penetrate the nest material and thus survive paraformaldehyde fumigation.

Observations made in the field on bee activity in paraformaldehyde-treated and untreated control nest material during July and August, 1989 indicated that fumigated nest material was clearly acceptable to leafcutting bees (Table VII). Initial rate of tunnel filling (mid-July) was actually higher in paraformaldehyde-treated material at all sites. Data collected near the end of the season (mid-August) show that percent available tunnels filled was greater in fumigated material than in control material at three of four sites. No unusual adult bee mortality was observed at any of the sites during the field season, and and analysis of developing leafcutting bee larvae in late August indicated that larvae reared in paraformaldehyde-treated nest material were as healthy as those reared in untreated control nest material.

Table VII. Percent available tunnels filled in paraformaldehyde treated and control untreated leafcutting bee nest material during the 1989 field season.

Site	Material	% available tunnels filled mid- July		mid- August	
		treated	control	treated	control
Lloydminster	Poly.Lam.01	24.3	17.6	62.6	53.3
Shellbrook	Poly.Lam.02	16.0	11.5	78.1	79.6
Zenon Park	Poly. Block	-a	-a	31.2	20.7
Birch Hills	Wood Laminate	61.1	51.3	87.4	86.3

a due to problem with alfalfa bloom, bees did not work at this site until after July 20, 1989.

Data on leafcutting bee sample quality, as determined by x-ray analysis of samples harvested in November, 1989, are presented in Table VIII. Cocoon quality was high at all sites, with no indication of prepupal mortality related to paraformaldehyde treatment of nest material. In fact, level of percent healthy prepupae was significantly higher in paraformaldehyde-treated nest material than in untreated control nest material at three sites.

Mean percent healthy prepupae was 84.2% in fumigated material, compared with 78.6% in control material. Mean percent combined larval and pollen mould was 1.75% in fumigated material, compared with 3.25% in control material. These data clearly indicate that fumigation of nest material with paraformaldehyde had no deleterious effect on leafcutting bee progeny reared in them.

Table VIII. Leafcutting bee sample quality as determined by x-ray analysis of leafcutting bee cocoons harvested from paraformaldehyde-treated and untreated control nest material from four test sites.

Nest Material Tested	Percent Healthy Prepupae[a]		Percent Combined Moulds	
	Paraform.	Control	Paraform.	Control
Poly.Lam.01	82.1a	72.5	1.4	1.7
Poly.Lam.02	86.8	85.6	5.5	8.8
Poly. Block	77.5a	69.7	0.1	0.0
Wood Laminate	90.3a	86.4	0.0	2.5
Mean values	84.2	78.6	1.75	3.25

[a] Percent paraform./healthy prepupae values followed by the letter "a" are significantly different from the corresponding control values at the 0.05 level.

Results of incubation tests carried out on healthy prepupae harvested from fumigated and control nest material from each field site are given in Table IX. Percent emergence data was similar between groups of incubated prepupae from fumigated and control nest material from all four sites. Furthermore, it was found that mean emergence time profiles for males, females, and combined sexes were uniform between fumigated and control nest material groups at each of the four sites.

Table IX. Mean percent emergence and time (days) to emergence in leafcutting bee cocoons harvested from paraformaldehyde-treated and untreated control leafcutting bee nest material.

Nest Material Tested		% Emergence	Mean Emergence Time Males	Females	Combined
Poly.Lam.01	Control	87.0	40.5	43.1	41.6
Poly.Lam.01	Treated	89.0	40.0	42.4	41.0
Poly.Lam.02	Control	100.0	40.9	42.8	41.6
Poly.Lam.02	Treated	99.0	41.6	42.5	42.0
Poly.Block	Control	100.0	42.7	44.7	43.8
Poly.Block	Treated	99.0	41.6	43.4	42.4
Wood Lam.	Control	99.0	39.5	41.1	40.0
Wood Lam.	Treated	98.0	38.3	41.3	39.7

Data for the additional paraformaldehyde fumigation test carried out under actual field conditions in a metal granary are presented in Appendix I. Analysis of samples indicates that significant control of mycoflora was obtained in polystyrene laminate and polystyrene block nest material, and in wood incubation trays. These data establish the efficacy of paraformaldehyde for control of microorganisms in polystyrene and wood material under field conditions. All nest material included in this experiment was subsequently placed in leafcutting bee shelters. This treated nest material was well-utilized by leafcutting bees during the 1989 field season.

E. Research on Other Leafcutting Bee Mortality

Mortality of alfalfa leafcutting bee larvae not apparently associated with overgrowth by pathogenic or saprophytic fungi (i.e. dead discoloured larvae/prepupae) was found at a level of 3.7% in the 1989/90 Saskatchewan Chalkbrood Survey and ranged from 0.7% to 17.1% in samples analysed (Appendix B). This incidence of larval mortality may be related not only to environmental factors (e.g. low temperatures), but also in some cases to the presence of fungal toxins in the nectar and pollen provisions on which developing larvae feed. Presence of toxins produced by Aspergillus or Saccharomyces spp. could cause feeding larvae to become weakened and susceptible to constipation. The combination of low temperatures and ingestion of toxic provisions would render elimination of fecal pellets extremely difficult for individuals thus affected; mortality and discolouration would result, but fungal growth would not likely occur.

Research undertaken to determine the effect of low temperatures on development of alfalfa leafcutting bees at the egg, larval, and prepupal stages has shown that mortality of eggs and early instar larvae is extremely high in the 6.0°C to 10.5°C range. Mid-instar larvae held at temperatures of 7°C and 10°C for 14 day periods prior to incubation at 28°C showed highest mortality as they reached the late instar defecating and cocoon-spinning stages. Late instar individuals subjected to cold treatments at the early defecating and late defecating stages showed highest mortality in the defecating and cocoon-spinning stages, respectively. Those individuals having completed cocoon-spinning or having entered prepupal diapause prior to cold treatment exhibited very low mortality. Analysis of additional data collected from experiments in which eggs, early instar, mid-instar, and late instar individuals were held at temperature treatments of 6.0, 10.5, 13.5, 18.5, 22.0, and 25.5°C prior to incubation at 28°C indicates that at temperatures in the 13.5°C to 25.5°C range, survival of individuals at all stages is high.

Research to determine the effects of two insect viruses on alfalfa leafcutting bee development was initiated during the 1988 field season. The two viruses, an entomopoxvirus (MsEPV) isolated from the migratory grasshopper and a baculovirus (McNPV) isolated from the bertha armyworm, may be used for future control of insect pests in areas where alfalfa leafcutting bees are in widespread use. For this reason, data on possible deleterious effects of these viruses towards the bees are useful.

Viable leafcutting bee eggs and early instar larvae were treated by micropipetting doses of baculovirus (10^4, 10^6 occlusion bodies) or entomopoxvirus (10^3, 10^5 occlusion bodies) onto the surface of the pollen/nectar provision.

Progression of development through egg and larval stages was uniform among treatments and mortality was confined to collapses in the egg stage. Control and treated individuals which successfully completed the cocoon-spinning stage and entered prepupal diapause were divided into two groups. The first group was held at a constant 28°C and adult emergence was monitored on a regular basis. The second group was held at 7°C for ca 130 days and then transferred to 28°C for incubation to determine percent successful adult emergence and mean emergence time.

Data indicated that overall emergence of treated leafcutting bees held at both temperature regimes was very similar to control emergence data. Mean emergence time (combined) for treated individuals was virtually identical to the combined control emergence time in the 28°C group (115.3 days) and actually lower than control emergence time in the 7°C/28°C group (treated - 36.6 days, control - 37.3 days). Percent emergence was 100% in all treatments for both incubation groups.

Representative control and treated individuals were set aside prior to the incubation test for analysis to determine the fate of ingested viral occlusion bodies. Fecal pellets from these cocoons were removed and ground with a sterile homogenizer prior to suspension in sterile 0.02% Tween 80. Material was then examined microscopically. No viral occlusion bodies were detected in control, MsEPV(10^3), MsEPV(10^5), and McNPV(10^4) treatment samples; however, examination of fecal material from bee larvae in the McNPV(10^6) treatment yielded a large number of undigested occlusion bodies, indicating survival and passage of these bodies through the gut of individuals consuming them. These data demonstrate that healthy alfalfa leafcutting bee larvae are able to consume , neutralize, and dispose of high doses of MsEPV and McNPV viral occlusion bodies with no detrimental effects.

Research to determine the effects of two insect-pathogenic fungi on alfalfa leafcutting bees was initiated during the 1989 field season. The two fungi, Beauveria bassiana and Aspergillus parasiticus, are fungal species which may be used for grasshopper control in the future. Data on possible deleterious effects of these fungi towards important pollinators such as the leafcutting bee are valuable; of special interest is the peroral pathway, where spores of these species are ingested by developing larval instars as they feed on nectar and pollen provisions.

In the bioassay experiments carried out, cells containing viable alfalfa leafcutting bee eggs and early instar larvae were removed from a trapnest and placed in sterile tissue culture plates following removal of the leaf cap. After a 24h observation period, healthy eggs and early instars were placed in control and treatment groups.

Treated individuals received doses of B.bassiana isolate 01, B.bassiana isolate 02, or A.parasiticus spores carried in 1.0ul droplets of sterile dH_2O. The microdroplets were pipetted onto the surface of the pollen/nectar provision. Feeding larvae were held at 25°C and observed on a daily basis to assess development and possible onset of pathological symptoms. Development from egg through early/mid-/late instar larval and larval defecating stages to completion of cocoon spinning was uniform among treatments (Table X). Mortality was generally confined to egg and early instar collapse which was not related to fungal pathogenicity. Only one incidence of advanced stage mortality was observed. This involved mortality in the third to fourth instar stage of an individual in the B.bassiana isolate 02 treatment group. Culture of the fungus growing on the cadaver indicated that an unrelated foliar mould was likely implicated.

Control and treated individuals which completed cocoon spinning were placed in two groups. The first group was held at 25°C until emergence was complete. The second group was transferred to 4°C storage until early January, 1990, when incubation at 25°C was initiated. Emergence was not complete in all treatments by late March, 1990, but preliminary data indicate that the alfalfa leafcutting bee larva is able to consume and dispose of spores from these two fungal species without suffering adverse effects.

Table X. Time required to complete cocoon spinning.

Treatment	(n)[a]	Mean time(days)	Range(days)
Control	24	11.8	8-15
B.b.01(10^3)	25	12.2	11-18
(10^5)	23	11.3	8-15
B.b.02(10^3)	23	11.7	8-13
(10^5)	23	11.3	8-13
A.par.(10^3)	24	11.6	8-15
(105)	23	11.6	8-18

[a] collapsing eggs were deleted from the experiment.

IV. DISCUSSION AND CONCLUSIONS

Surveys of Saskatchewan leafcutting bee populations from 1988 and 1989 field seasons have indicated that chalkbrood disease (Ascosphaera aggregata) is present in the province at very low levels. Chalkbrood infestations of up to 27.8% have been reported in southern Alberta bee populations during the same two-year period, and thus the spread of the disease remains a major concern among Saskatchewan alfalfa seed producers. The number of producers participating in the 1989/90 chalkbrood survey was higher than in any previous year, and the demand for information on chalkbrood and other fungal problems in leafcutting bees continues. Levels of other Ascosphaera species (A.larvis, A.pollenicola, and A.variegata) have remained constant over the five year period of the survey; these other Ascosphaera species appear to be primarily saprophytic in nature and are not a primary cause of leafcutting bee mortality.

Fungal species referred to collectively as foliar moulds are far more prevalent in Saskatchewan leafcutting bee populations than Ascosphaera species. Surveys of cocoon samples from numerous leafcutting bee populations throughout province inducate that the most common foliar moulds found on larval cadavers, in contaminated nectar and pollen provisions, and on cocoon surfaces are Aspergillus, Eurotium/Penicillium, and Rhizopus species. The most common yeast, Trichosporonoides species, is associated with all leafcutting bee populations sampled. Bacteria usually isolated from leafcutting bee samples include Enterobacter, Bacillus, and Pseudomonas species. The presence of these foliar moulds, yeasts, and bacteria may disrupt normal larval development and cause egg or larval mortality, often leading to complete overgrowth of the cocoon.

The presence of foliar moulds in leafcutting bee populations poses problems not only for the bees, but for the health of producers as well. Alfalfa seed producers, working in close proximity to leafcutting bees and equipment on a regular basis, are exposed to high levels of spores from leafcutter bee-related fungi at a time when occurrence of invasive fungal disease is being recognized as a major medical problem. Discussions with producers often bring to light anecdotal evidence of health problems, generally allergic or asthmatic in nature, which seem to be related to contact with leafcutting bees or cocoons containing diapausing prepupae.

Individuals working with leafcutting bees should observe basic safety precautions, particularly during bee incubation and harvesting operations, when large numbers of cocoons or emerging bees concentrated in confined areas lead to high levels of fungal spores in the immediate environment. The use of efficient ventilation systems and appropriate protective equipment, combined with safe work habits, can minimize contact with potentially harmful spores. Work clothes, dust masks, and gloves should be worn to minimize movement of contamination into the living environment. A respirator or positive pressure air filtration system may be used if a known sensitivity to foliar moulds exists. Eating, smoking, and drinking should be done away from the work area, and hands washed and work clothes removed when leaving the area. Facilities used for cocoon incubation and harvesting should be cleaned and disinfected following use.

In addition to the safety precautions discussed above, incorporation of foliar mould control techniques has been shown to be extremely effective in reducing mould levels. Use of sodium hypochlorite bleach dipping and kiln-heating can virtually eliminate microorganisms in polystyrene and wood nest material, while bleach dipping of cocoons prior to incubation will lower spore levels in the incubator and substantially reduce spore numbers carried by emerging leafcutting bees.

Research reported here on the use of paraformaldehyde as an agent for the control of foliar moulds, yeasts, and bacteria associated with alfalfa leafcutting bee populations has clearly indicated that paraformaldehyde fumigation is a highly efficaceous alternative compound for control of foliar moulds in leafcutting bee nest material. Data indicate that leafcutting bees will readily accept paraformaldehyde-treated nest material in the field; as well, it has been demonstrated that leafcutting bee prepupae harvested from fumigated nest material are as viable as those harvested from untreated control nest material. The cost of paraformaldehyde fumigation (estimated to be less than $ 0.10 per nest box) is significantly lower than the cost of bleach dipping (calculated to be $ 0.50 per nest box). Based on the research data collected on paraformaldehyde efficacy, an application has been made to the Pesticides Directorate of Agriculture Canada for a minor use registration of the fumigant paraformaldehyde for control of foliar moulds in leafcutting bee nest material through the Minor Use of Pesticides Program.

More comprehensive use of foliar mould control techniques by producers will reduce levels of mycoflora in leafcutting bee populations and result in increased population viability. Healthier bee populations will enhance the value of Saskatchewan leafcutting bees and increase their competitive edge in the world export market.

APPENDIX A

1988 Saskatchewan Chalkbrood Survey Data

CATEGORY	STATISTIC (%) Mean	St.Dev.	High	Low
HEALTHY PREPUPAE	79.6	8.3	91.9	41.8
DEAD LARVAE/PREPUPAE				
Discoloured	3.9	2.6	15.8	1.1
Mouldy	0.24	0.25	1.52	0.0
Ascosphaera aggregata	0.002	0.015	0.13	0.0
Other Ascosphaera spp.	0.004	0.024	0.16	0.0
MOULDY COCOONS				
Pollen Mould	2.27	2.34	11.87	0.0
Pollen Ascosphaera spp.	0.52	1.03	6.03	0.0
PARASITES	2.1	3.4	20.7	0.0
SECOND GENERATION	0.6	0.7	3.4	0.0
POLLEN BALLS	9.3	4.7	38.9	4.2
CRUSHED COCOONS	1.3	1.7	9.1	0.0
OTHER	0.2	0.3	1.1	0.0
LIVE COUNT / LB	4083	498	5170	1968
LIVE COUNT / KG	8991	1096	11386	4334

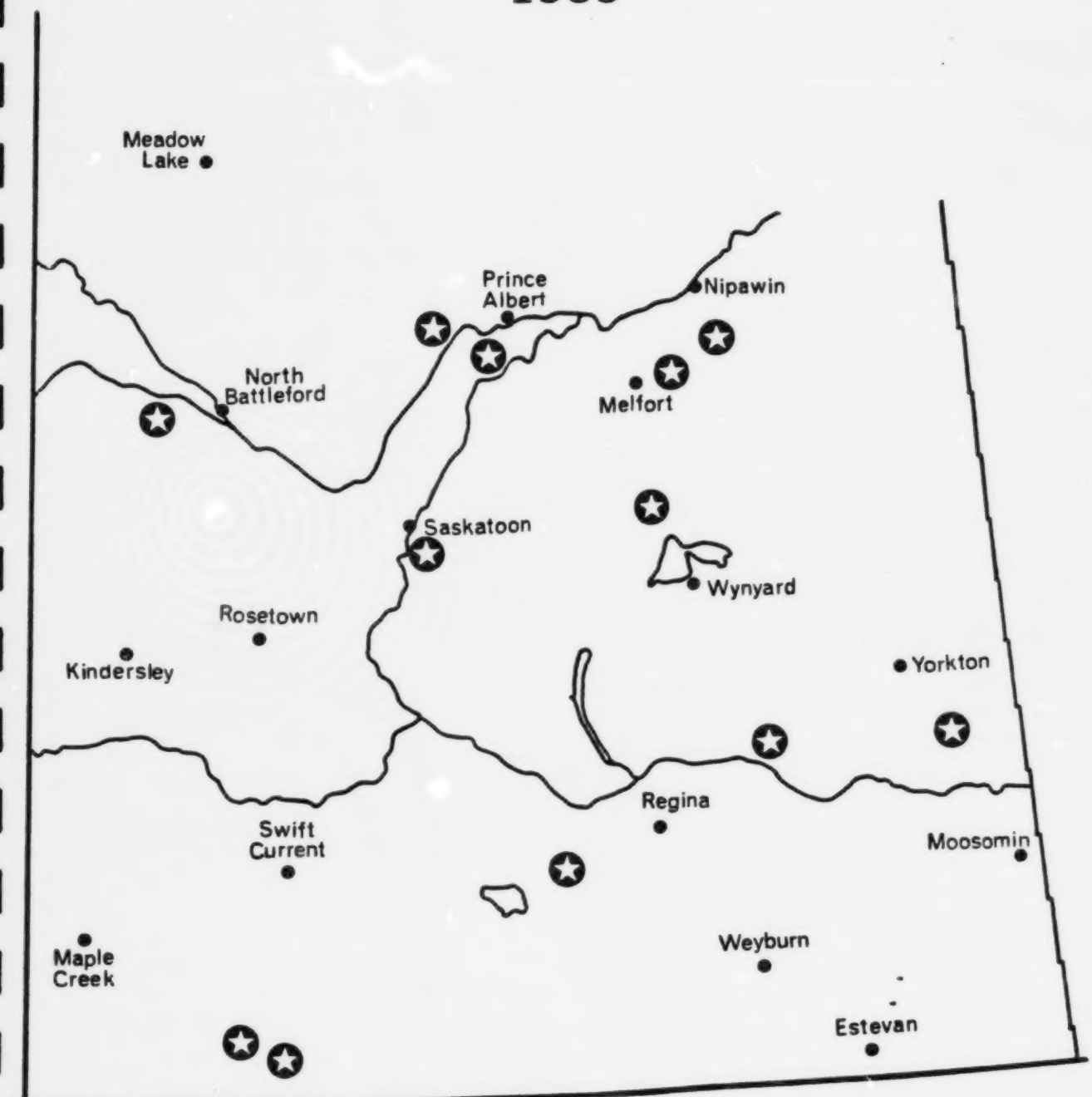
APPENDIX B

1989 Saskatchewan Chalkbrood Survey Data

CATEGORY	STATISTIC (%) Mean	High	Low
HEALTHY PREPUPAE	79.4	92.5	54.0
DEAD LARVAE/PREPUPAE			
Discoloured	3.7	17.1	0.7
Mouldy	0.25	2.65	0.00
Ascosphaera aggregata	0.00	0.00	0.00
Non-spor.Chalkbrood	0.009	0.56	0.00
Other Ascosphaera spp.	0.007	0.15	0.00
MOULDY COCOONS			
Pollen Mould	2.81	19.07	0.00
Pollen Ascosphaera spp.	0.56	8.07	0.00
PARASITES	1.77	12.72	0.00
SECOND GENERATION	0.9	9.0	0.0
POLLEN BALLS	8.9	28.3	3.5
CRUSHED COCOONS	1.5	8.8	0.0
OTHER	0.3	1.7	0.0
LIVE COUNT/LB.	4089	4807	2937
LIVE COUNT/KG.	9004	10585	6468

APPENDIX C

SASK. TRAP NEST LOCATIONS 1989



APPENDIX D

Mycoflora isolated from Alfalfa Leafcutting Bee Populations in Saskatchewan

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MOULDS

- *Alternaria alternata*
- *Ascosphaera aggregata*
- *Ascosphaera atra*
- *Ascosphaera larvis*
- *Ascosphaera pollenicola*
- *Ascosphaera variegata*
- *Aspergillus glaucus*
- *Aspergillus niger*
- *Cylindrocarpon* sp.
- *Eurotium chevalieri*
- *Mucor* sp.
- *Penicillium purpurogenum*
- *Penicillium simplicissimum*
- *Penicillium spinulosum*
- *Penicillium* sp.
- *Rhizopus oryzae*
- *Trichoderma citrinoviride*
- *Trichothecium roseus*
- *Ulocladium* sp.

YEASTS

- *Saccharomyces* sp.
- *Trichosporonoides* sp.

BACTERIA

- *Bacillus circulans*
- *Bacillus mycoides*
- *Bacillus* sp.
- *Corynebacterium* sp.
- *Enterobacter agglomerans*
- *Flavobacterium breve*
- *Pseudomonas solanacearum*
- *Pseudomonas* sp.

APPENDIX E

Paraformaldehyde Treatment of Leafcutting Bee Nest Material

Experimental Protocol : Fumigation of nest material and assessment of efficacy for control of mycoflora within nest tunnels.

1. Place nest material in fumigation chamber and condition room for 48 hours at 25° C with a relative humidity of 60 - 70 %.

2. Sample tunnels pre-treatment using sterile cotton-tipped wood applicators wetted with sterile distilled water; transfer applicators to sterile culture tubes as samples are taken. Move samples to laboratory as soon as possible; reconstitute individual samples under sterile conditions (2 mL sterile distilled water per sample), vortex ca 15 sec., and culture as 0.1 mL aliquots on PDA (potato dextrose agar) media. Incubate cultures at ca 28° C and assess at 24 hour intervals.

3. Treat nest material with paraformaldehyde at a rate of ca 20 grams/m^3 by placing product in a heat generation sublimation unit attached to a timer. Set sublimation unit to maximum heat, set timer to provide power for 4.0 hours, then leave chamber, seal, lock, and post it. From onset of treatment time, allow a 24 hour period for formaldehyde gas to dissipate and a further 24 hour period for filtered ventilation.

4. Open and re-enter chamber wearing full body protective clothing and appropriate face and respiratory protection. Sample tunnels post-treatment using the technique outlined in section 2. above, culture samples collected, and compare pre- and post-treatment results.

5. Allow nest material to air briefly following ventilation of isolation chamber, place in sealed plastic bags for transport to the field, and deploy in shelters for comparison with control material.

APPENDIX F

Efficacy of paraformaldehyde fumigation of leafcutting bee nest material (20.9 g/m³) in an isolation chamber, 1989.[a]

Type of material	Microorganism	No. cfu pre-treat.	No. cfu post-treat.	% control
Poly. Laminate 01	Moulds	1642[b]	45	97.3
	Yeasts	5850	2	99.9
	Bacteria	643	24	96.3
	Total	8135	71	99.1
Poly. Laminate 02	Moulds	4401[b]	30	99.3
	Yeasts	1122	0	100.0
	Bacteria	505	47	90.7
	Total	6028	77	98.7
Poly. Block	Moulds	291[b]	71	75.6
	Yeasts	180	12	93.3
	Bacteria	2815	27	99.0
	Total	3286	110	96.7
Wood Laminate	Moulds	273[b]	64[b]	76.5
	Yeasts	2093	65	96.9
	Bacteria	988	323	67.3
	Total	3354	452	86.5

[a] Data presented as combined mean cfu (colony-forming units) per 0.1 mL aliquot plated.
[b] Samples also contained an indeterminate number of Rhizopus cfu.

APPENDIX G

Mould, yeast, and bacterial species identified in pre-treatment and post-treatment cultures of samples taken from tunnels in polystyrene laminate leafcutting bee nest material before and after paraformaldehyde fumigation at a rate of 20 g/m³.

Microorganism	Poly.Laminate 01 cfuPRE	Poly.Laminate 01 cfuPOST	Poly.Laminate 02 cfuPRE	Poly.Laminate 02 cfuPOST
Alternaria alternata	1	2	-	-
Aspergillus niger	30	-	38	(19)
Eurotium/Penicillium complex	1611	40	4362	3
Mucor sp.	-	1	-	-
Rhizopus oryzae	a	-	b	-
Trichoderma citrinoviride	-	-	1	-
Unidentified mould spp.	-	2	-	8
Trichosporonoides sp.	5850	1	1122	-
Unidentified yeast spp.	-	1	-	-
Bacillus spp.	122	5	4	13
Corynebacterium spp.	-	1	-	5
Enterobacter agglomerans	510	11	500	21
Flavobacterium breve	-	-	-	1
Pseudomonas spp.	10	3	1	7
Unidentified bacterial spp.	1	4	-	-

a *Rhizopus oryzae* present in 100% of samples cultured.
b *Rhizopus oryzae* present in 25% of samples cultured.

APPENDIX H

Mould, yeast, and bacterial species identified in pre-treatment and post-treatment cultures of samples taken from tunnels in polystyrene block and wood laminate leafcutting bee nest material before and after paraformaldehyde fumigation at a rate of 20 g/m^3.

Microorganism	Poly.Block cfuPRE	Poly.Block cfuPOST	Wood Laminate cfuPRE	Wood Laminate cfuPOST
Alternaria alternata	-	1	-	-
Aspergillus niger	121	1	15	1
Eurotium/Penicillium complex	170	66	255	60
Mucor sp.	-	-	-	-
Rhizopus oryzae	a	-	b	c
Trichoderma citrinoviride	-	-	-	-
Unidentified mould spp.	-	3	3	3
Trichosporonoides sp.	180	11	2093	65
Unidentified yeast spp.	-	1	-	-
Bacillus spp.	65	3	112	105
Corynebacterium spp.	-	-	25	-
Enterobacter agglomerans	2750	6	850	209
Flavobacterium breve	-	1	-	-
Pseudomonas spp.	-	14	-	9
Unidentified bacterial spp.	-	3	1	-

a Rhizopus oryzae present in 75.0% of samples cultured.
b Rhizopus oryzae present in 25.0% of samples cultured.
c Rhizopus oryzae present in 37.5% of samples cultured.

APPENDIX I

Efficacy of paraformaldehyde fumigation of leafcutting bee nest material and wood bee incubation trays at a rate of 20.0 g/m³ under actual field conditions, 1989.[a]

Type of material	Microorganism	No. cfu pre-treat.	No. cfu post-treat.	% control
Poly. Laminate	Moulds	68[b]	1	98.5
	Yeasts	0	0	-
	Bacteria	4	0	100.0
	Total	72	1	98.6
Poly. Block	Moulds	25[b]	1	96.0
	Yeasts	3	0	100.0
	Bacteria	250	0	100.0
	Total	278	1	99.6
Wood Incubation Trays	Moulds	121[b]	0	100.0
	Yeasts	85	0	100.0
	Bacteria	500	64	87.2
	Total	706	64	90.9

[a] Data presented as combined mean cfu (colony-forming units) per 0.1 mL aliquot plated.
[b] Samples also contained an indeterminate no. of Rhizopus cfu.

Project 2.3-13
"Control of Chalkbrood and Other
Fungal Problems in Leafcutting Bees"

Practical information and recommendations for
alfalfa seed producers and extension personnel

Surveys of Saskatchewan leafcutting bee populations from the 1988 and 1989 field seasons have indicated that chalkbrood disease (Ascosphaera aggregata) is present in the province at very low levels. However, other mould species referred to as foliar moulds are present at much higher levels in leafcutting bee populations. They are found in leafcutting bee nest material and on cocoon surfaces, pollen balls, working adult bees, and larval cadavers. While these moulds are generally saprophytic, that is, growing on any material which will provide them with nutrients (leaf debris, pollen provisions, dead bee larvae), some species of foliar moulds may cause considerable damage to bee populations.

Bacteria and yeasts are capable of fermenting the nectar and pollen provision on which the bee egg is laid; this may lead directly to egg or larval mortality and subsequently allow foliar moulds to overgrow the entire cocoon. The result is a mouldy pollen ball contaminated with a number of mould and yeast species. Larvae which die for other reasons may also become overgrown with foliar moulds. Research has shown that the percentage of healthy larvae is significantly reduced in populations where foliar moulds are present at high levels on cocoon surfaces.

Many of the foliar moulds found in association with leafcutting bee populations are potentially harmful not only to the bees, but to alfalfa seed producers as well. Alfalfa seed producers, working in close proximity to leafcutting bees and cocoons on a regular basis, are exposed to high levels of spores from bee-related fungi at a time when fungal disease is becoming recognized as a major medical problem. Some of the foliar moulds isolated from Saskatchewan leafcutting bee populations have been implicated in allergic reactions and bronchopulmonary disease and are considered major fungal allergens. Individuals working with bees should observe basic safety precautions, particularly during bee incubation and harvesting operations. At these times, large numbers of cocoons or emerging bees concentrated in confined areas may lead to high levels of airborne fungal spores in the workplace. The use of efficient ventilation systems and appropriate protective equipment, combined with safe work habits, can minimize contact with potentially harmful spores.

Work clothes, dust masks, and gloves should be worn to minimize movement of contamination into the living environment. A respirator or positive pressure air filtration system may be used if a known sensitivity to foliar moulds exists. Eating, smoking, and drinking should be done away from the work area, and hands washed and work clothes removed when leaving the area. Facilities used for cocoon incubation and harvesting should be cleaned and disinfected following use.

Incorporation of decontamination techniques such as bleach dipping and heat treatment of leafcutting bee nest material has been shown to be highly effective in reducing levels of foliar moulds, as well as yeasts and bacteria.

Dipping of nest material in sodium hypochlorite bleach is most compatible with those operations utilizing polystyrene and styrofoam nest materials, since wood material may warp if not properly dried following wetting. Nest material should be dipped in a 3 to 5% active chlorine solution for 3 to 5 minutes. A bleach test kit should be used periodically to determine the active chlorine concentration of the dipping solution. A wetting compound (for example Triton X-100, Agral 90, or Amway LOC) can be added to the dipping solution at a rate of 0.1% to enhance penetration of bleach solution into nest tunnels. After nest material has been dipped it should be well drained and then either stacked to dry or placed in field shelters.

Heat treatment within a temperature range of 80°C to 95°C for a 12 hour period is required in order to eliminate foliar moulds, yeasts, and bacteria from wood laminate and wood block nest material. It is critical to maintain a uniform temperature within the treatment facility during the heat treatment procedure. Fans may be used to circulate hot air through the kiln or oven, and nest boxes should be stacked loosely to allow for movement of hot air through the material.

Research on the fumigant paraformaldehyde has indicated that it may be a highly efficaceous alternative compound for control of foliar moulds in leafcutting bee nest material. Paraformaldehyde is not currently registered for use in leafcutting bee operations; based on efficacy data collected during the past two field seasons, an application has been made to the Pesticides Directorate of Agriculture Canada for a minor use registration of paraformaldehyde for control of foliar moulds in leafcutting bee nest material under the Minor Use of Pesticides Program.